BULLETIN OF THE
U.S. DEPARTMENT OF AGRICULTURE

No. 164

Contribution from the Bureau of Soils, Milton Whitney, Chief.
January 30, 1915.

(PROFESSIONAL PAPER.)

FIELD TEST WITH A TOXIC SOIL CONSTITUENT: VANILLIN.

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INTRODUCTION.

The presence of vanillin in soils and in a number of plants has led to a study of its effect on growth. Its harmful effect on wheat plants in water and nutrient culture solutions has been demonstrated, while the experiments reported in this paper deal with its effect in soils on crops grown in the field and in pots in the greenhouse.

Until recently vanillin had not been definitely isolated or identified in soils, but much information had been obtained in the work of this laboratory to indicate its presence in a number of soils. The isolation of vanillin in crystal form from certain soils and its definite identification has now been accomplished [1] and its effect on soil fertility has become an interesting subject for investigation.

Vanillin has been reported in the seeds and roots of oats,[2] seeds of white lupine,[3] asparagus shoots,[4] in raw-beet sugar,[5] and in the leaves and roots of a number of other plants. It has recently been reported to occur in rotten oak wood, in pineapples, in lawn grass, in ungerminated wheat, in wheat bran, in the roots, tops, and seeds of wheat seedlings, and in water in which wheat seedlings grew.[6] Its presence in wood and various forms of vegetation has led to the conclusion that vanillin in soils has its origin in vegetable débris.

Vanillin has the characteristics of an aldehyde, and, like the salicylic aldehyde already reported,[7] is toxic to plants, though to a less degree.

[1] Shorey, E. C., J. Agr. Res. **1**, 357 (1914).
[2] de Routon, Compt. rend., **125**, 797 (1897).
[3] Campani and Grimaldi, Chem. Centr., **1**, 377 (1888).
[4] Von Lippmann, Ber. Chem. Ges., **18**, 3335 (1885).
[5] Scheibler, Ber. Chem. Ges., **13**, 335 (1880) Lippmann, ibid., 662.
[6] Sullivan, M. X., Jour. Indus. and Eng. Chem., **6**, 119 (1914).
[7] Schreiner, O., and Skinner, J. J., Bul. 108, U. S. Department of Agriculture, 1914.

NOTE.—The effect upon plant growth of vanillin, a toxic soil constituent, as demonstrated in pot experiments and field tests, is described in this bulletin.

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It is harmful to wheat seedlings in water cultures, even in such low concentrations as a few parts per million, and the plants are killed in solutions of 500 parts per million in a few days.[1] The toxic effect is less marked upon the tops of the wheat plants than upon their roots. Vanillin is also harmful in nutrient culture solutions composed of calcium acid phosphate, sodium nitrate, and potassium sulphate. It is an oxidizable substance and is less harmful in solutions of some of these nutrient salts than in others, especially those high in nitrate.[2] Sodium nitrate and calcium carbonate,[3] which themselves induce oxidation, ameliorate the harmfulness of vanillin.

The isolation of vanillin from soils and its harmfulness to plants in aqueous solutions has made a study of its effect in soils and under field conditions essential. The results of such experiments with cowpeas, garden peas, and string beans will now be given, together with the action of vanillin on clover in soil in pots and with wheat plants grown in several soils of different characters.

EFFECT OF VANILLIN ON CLOVER IN POTS.

An experiment to determine the effect of vanillin on clover was made by growing clover in Chester loam soil in large pots. Ordinary clay flower pots holding 6 pounds of soil were used. One pot was untreated; the other had a total of 300 parts per million of the vanillin added to it.

When the soil was potted, 100 parts per million of the vanillin was added and clover then sown, 0.5 gram of seed per pot. The clover was sown April 12, and came up well. On April 28, 50 parts per million of vanillin were added in solution through a funnel passing into the soil nearly to the bottom of the pot, thus avoiding direct contact with the tops or roots of the clover. On May 15 another 50 parts per million were added, and on June 1 and June 10, 50 parts per million were added, making the total application 300 parts per million. The experiment was discontinued June 21, 1912. The effect of vanillin was noticeable from the first.

The harmful effect of the vanillin is shown by comparing the untreated pot and the vanillin-treated pot shown in Plate I. The vanillin-treated plants were healthy in appearance but stunted in growth.

The green weight taken at the termination of the experiment was 8 grams from the untreated pot and only 3.8 grams from the vanillin-treated pot, a decrease of 53 per cent.

The soil used in this experiment was a soil of moderate productiveness, and vanillin applied to it at different periods of the growth of the plants was distinctly harmful. Other experiments were made to

[1] Schreiner, Reed, and Skinner, Bul. 47, Bureau of Soils, U. S. Dept. Agr. (1908).
[2] Schreiner and Skinner, Bul. 77, Bureau of Soils, U. S. Dept. Agr. (1911).
[3] Schreiner and Reed, Am. Chem. Soc., 30, 85 (1908).

test the effect of different amounts of vanillin in several soils, each having different properties and being of different geological origin. In the following experiments wheat was used as the test crop and the total application of vanillin was made before the soil was potted and seeds planted.

EFFECT OF VANILLIN ON WHEAT IN POTS.

In this experiment the effect of vanillin in several soils was studied by growing wheat in pots. The soils used were infertile Florida sand, an infertile sample of Susquehanna sandy loam, and a good sample of Hagerstown loam. The paraffined wire pot method [1] was used, six wheat plants were grown in each pot, and two pots were used for each treatment. The plants grew from May 5 to May 24. Photographs of the growing plants were taken, which show the action of vanillin in each soil. At the end of the experiment the green weight was determined.

The Florida sand used in this experiment had grown citrus fruits in the field and was unproductive. A laboratory examination showed the soil to be acid. Vanillin was isolated from this soil in the investigations referred to above. The Susquehanna sandy loam was taken from an infertile area in Maryland. The natural growth on this soil was poor, and its response to fertilizer and cultural treatments was only moderate. Its oxidizing power and life activities were found to be very weak. The Hagerstown loam is a fertile soil. The soil was taken from a productive field of the Pennsylvania Agricultural Experiment Station. The soil is neutral in reaction, has strong oxidizing power, and grows thrifty plants in pots.

Vanillin was used in amounts of 100 to 500 parts per million. It was applied to the soil by dissolving in water and mixing the solution in the soil before potting. The results of the experiment on the effect of vanillin in the Florida sand, Susquehanna sandy loam, and Hagerstown loam are given in Table I. The actual green weight of the plants grown in the two pots are given for each treatment and the relative weight with the growth in the untreated soil taken as 100.

TABLE I.—*Effect of vanillin on wheat plants in pots grown in Florida sand, Susquehanna sandy loam, and in Hagerstown loam.*

Treatment.	Florida yellow sand (infertile sand).		Susquehanna sand loam (unproductive soil).		Hagerstown loam (productive soil).	
	Green weight.	Relative weight.	Green weight.	Relative weight.	Green weight.	Relative weight.
	Grams.		*Grams.*		*Grams.*	
Soil untreated	1.40	100	1.80	100	1.98	100
Soil + 100 p. p. m. vanillin	1.32	94	1.85	103	1.87	94
Soil + 200 p. p. m. vanillin	1.32	94	1.70	94	2.02	102
Soil + 300 p. p. m. vanillin	1.35	98	1.33	74	2.05	103
Soil + 400 p. p. m. vanillin	1.20	86	1.30	72	1.96	99
Soil + 500 p. p. m. vanillin	1.18	84	1.02	57	1.95	99

[1] Cir. 18, Bureau of Soils.

The vanillin was quite harmful in amounts of 400 and 500 parts per million in the Florida sand and was only moderately harmful in amounts of 100 to 300 parts per million. With the Susquehanna sandy loam the vanillin reduced growth considerably when applied at the rate of 300, 400, and 500 parts per million. It was slightly harmful with 100 and 200 parts per million. Vanillin had no harmful effect in the Hagerstown loam—two of the treatments were slightly above the check and three slightly below. The growth in the untreated soil of the Hagerstown loam was better than in the Susquehanna sandy loam and considerably better than in the Florida sand. The effect of vanillin in the three soils is shown in Plate II.

It is seen from this experiment that vanillin is harmful in two of the soils and has no effect in the third. Vanillin is easily oxidized and changed under favorable conditions, and if this took place the action on plant growth would not be noticeable. The Florida sand was found to contain vanillin when sent in from the field and, as would be expected, added quantities of vanillin would not be changed and it would remain as such to have its effect on plants grown in the soil. The Susquehanna sandy loam is also a soil having small oxidizing power and low life activity, and added quantities of vanillin apparrently remained as such and had their effect on plant growth. The Hagerstown loam is a soil of entirely different characteristics, being highly productive, which indicates good life activities and good oxidizing power. Vanillin when added does not have harmful effects on plants grown in the soil, as it probably does not remain in this soil as such, but is changed or destroyed by the oxidation which is going on in soils of this character.

In order to study further the action of vanillin in soils and its bearing on soil fertility, the effect of vanillin under field conditions was tested in plots. Three leguminous crops—cowpeas, string beans, and garden peas—were grown to maturity in this experiment, with the following results:

EFFECT OF VANILLIN ON COWPEAS, STRING BEANS, AND GARDEN PEAS GROWN IN THE FIELD.

The effect of vanillin in soils under field conditions was tested on plots at the experiment farm of the Agricultural Department at Arlington, Va. Three crops were grown, namely, cowpeas, string beans, and garden peas. These experiments were made during the summer of 1913. The treated plot was adjoined on each side by an untreated plot growing the same crop. Each plot was $8\frac{1}{4}$ feet square, or one-fourth of a square rod; that is, one six hundred and fortieth of an acre.

EFFECT OF VANILLIN ON CLOVER.

(No. 1, untreated; No. 2, vanillin.)

FIG. 1.—EFFECT OF VANILLIN ON WHEAT IN FLORIDA SAND.

(No. 1, Soil untreated; No. 2, vanillin 100 p. p. m.; No. 3, vanillin 200 p. p. m.; No. 4, vanillin 300 p. p. m.; No. 5, vanillin 400 p. p. m.; No. 6, vanillin 500 p. p. m.)

FIG. 2.—EFFECT OF VANILLIN ON WHEAT IN SUSQUEHANNA SANDY LOAM.

(No. 1, Soil untreated: No.2, vanillin 100 p. p. m.; No. 3, vanillin 200 p. p. m., No. 4, vanillin 300 p. p. m.: No. 5, vanillin 100 p. p. m.; No. 6 vanillin 500 p. p. m.)

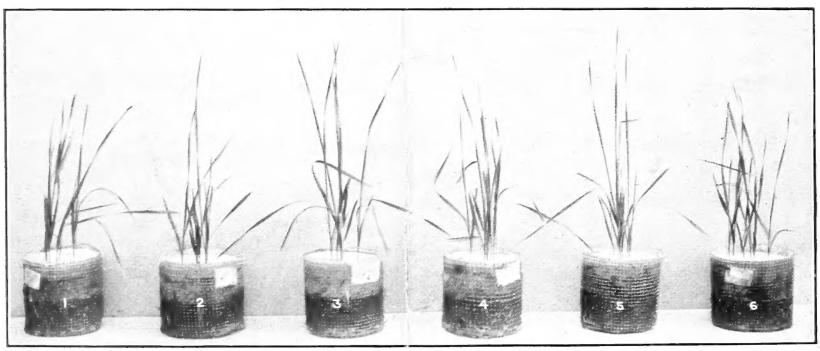

FIG. 3.—EFFECT OF VANILLIN ON WHEAT IN HAGERSTOWN LOAM.

(No. 1, Soil untreated; No. 2, vanillin 100 p. p. m.; No. 3, vanillin 200 p. p. m.; No. 4, vanillin 300 p. p. m.; No. 5, vanillin 400 p. p. m.; No. 6, vanillin 500 p. p. m.)

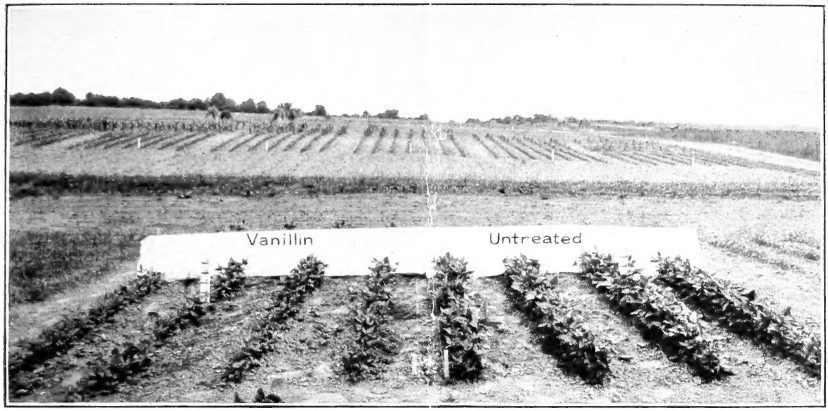

FIG. 1.—EFFECT OF VANILLIN ON COWPEAS IN THE FIELD.

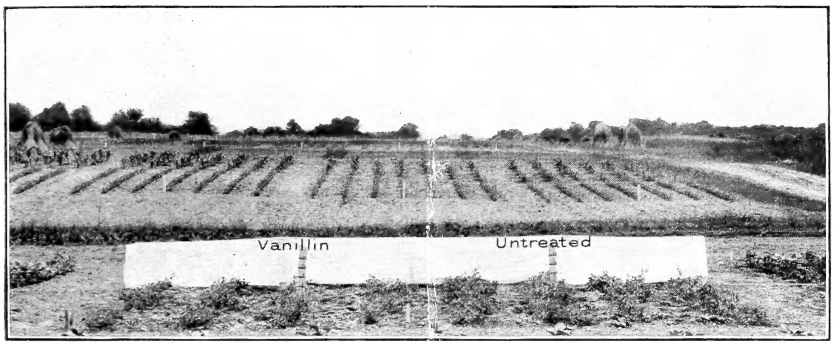

FIG. 2.—EFFECT OF VANILLIN ON GARDEN PEAS IN THE FIELD.

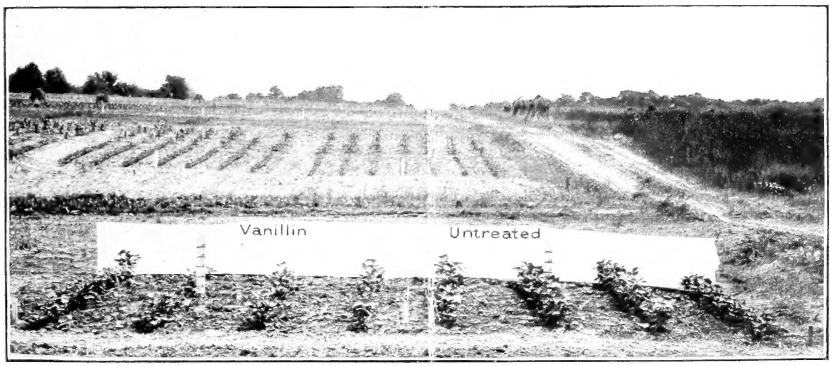

FIG. 3.—EFFECT OF VANILLIN ON STRING BEANS IN THE FIELD.

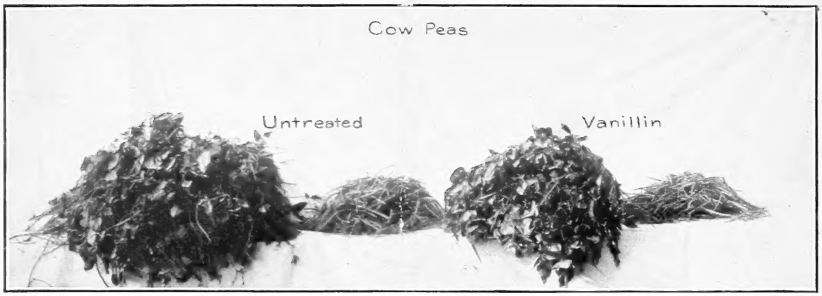

FIG. 1.—YIELD OF COWPEAS, VINES, AND PODS ON CHECK PLOT *a* AND ON VANILLIN-TREATED PLOT.

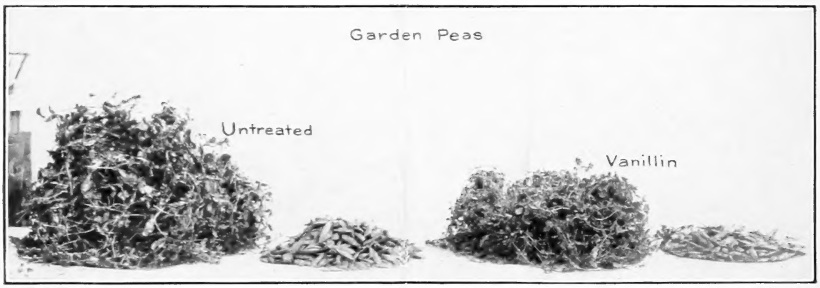

FIG. 2.—YIELD OF GARDEN PEAS, VINES, AND PODS ON CHECK PLOT *a* AND ON VANILLIN-TREATED PLOT.

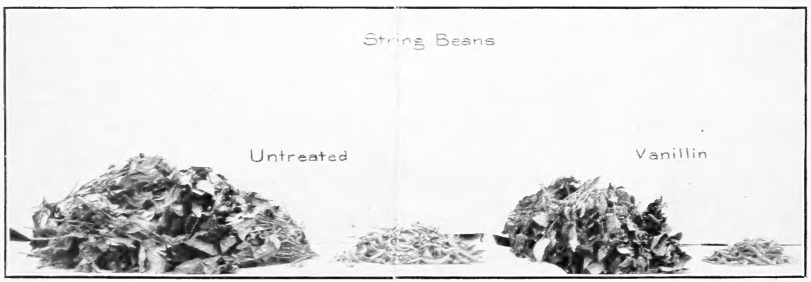

FIG. 3.—YIELD OF STRING BEANS, VINES, AND PODS ON CHECK PLOT *a* AND ON VANILLIN-TREATED PLOT.

The soil on which these experiments were made is a silty clay loam, low in organic matter. The ground is level and has surface drainage. The soil throughout these plots and their controls is uniform, so the results secured should not be considered as unduly influenced by irregularities due to nonuniformity of the soil in different plots. The soil is of an acid nature. The land was plowed early in May and prepared for seeding.

Four applications of vanillin were made. The first on May 20, one day before the planting of seed. The other three applications were made periodically during the growth of the crops—May 28, June 5, and June 24. The vanillin was applied by dissolving in water, sprinkling the solution uniformly on the surface of the ground before planting, and raking the soil thoroughly. The remaining applications were made after planting by sprinkling the solution between the rows of plants, the soil being subsequently cultivated. The total application was at the rate of 285 pounds per acre, in four equal parts.

The crops germinated uniformly. The effect of the vanillin was noticeable from the beginning and throughout the experiment. The growth was stunted, though the plants grew slowly to maturity, and were harvested.

EFFECT OF VANILLIN ON COWPEAS.

The cowpeas were sown May 21, 1913, the plots having been previously prepared, and were harvested September 7, 1913. The plants in the untreated plots made more vigorous growth and had a better color than those in the vanillin-treated plot. The vanillin-treated plants had a pale-green color and grew slenderer than those on the untreated plot. The appearance of the plants on June 27 is shown in Plate III, figure 1. The four rows of plants growing on the left are on the vanillin-treated plot, and the four rows on the right on the untreated plot. The picture shows that at this stage of growth the vanillin has greatly affected the cowpeas. This effect was even more marked as the crop approached maturity. When mature the peas were picked from the vines and weighed. The weight of the cowpea vines was taken, and after drying the weight of the cured hay was also determined. In Plate IV, figure 1, are shown the vines and pods as taken from the untreated and treated plots. The effect of the vanillin in depressing yield is here also apparent.

In Table II are given the yields obtained in this experiment with vanillin and cowpeas. The weight of vines and pods is given as obtained from the individual plots and also in terms per acre.

TABLE II.—*Yield of cowpeas as affected by vanillin in the field.*

Treatment.	Yield per plot.			Yield per acre.		
	Vines.		Pods.	Vines.		Pods.
	Green.	Cured.		Green.	Cured.	
	Pounds.	*Pounds.*	*Pounds.*	*Tons.*	*Tons.*	*Tons.*
Check *a*	28.0	10.0	6.6	8.96	3.20	2.11
Check *b*	23.0	8.5	5.6	7.36	2.72	1.79
Average check	25.5	9.3	6.1	8.16	2.96	1.95
Vanillin	17.0	5.7	4.0	5.44	1.82	1.27
Difference	8.5	3.6	2.1	2.72	1.14	.68

From the table it is seen that the average production of the two check plots was 8.16 tons per acre of green pea vines, or 2.96 tons per acre of cured hay, while the vanillin plot produced only 5.44 tons per acre of green vines, or 1.82 tons per acre of cured hay. This is a reduction of 2.72 tons per acre of green vines, or 1.14 tons per acre of cured hay due to the vanillin, a reduction of 33 per cent of green vines and 39 per cent of cured hay. The average production of the two check plots was 1.95 tons per acre of pods, while the vanillin plots produced 1.27 tons per acre, a reduction of 35 per cent.

EFFECT OF VANILLIN ON GARDEN PEAS.

The garden peas were sown in the untreated and vanillin-treated plots May 21, the germination was good, and a good stand was obtained. The vanillin checked the growth of the peas from the start and the difference was pronounced throughout the entire period of growth. The crop was harvested June 30; the vines and peas were weighed separately. The appearance of the plants in the early stages of their growth is shown in Plate III, figure 2. The weights and measurements of vines and peas are given in Table III.

TABLE III.—*Yield of garden peas as affected by vanillin in the field.*

Treatment.	Yield per plot.			Yield per acre.		
	Vines.	Peas.		Vines.	Peas.	
	Pounds.	*Pounds.*	*Pints.*	*Pounds.*	*Pounds.*	*Pecks.*
Check *a*	1.72	1.66	4.50	1,101	1,062	180
Check *b*	1.50	1.48	4.0	950	947	160
Average check	1.61	1.57	4.25	1,030	1,004	170
Vanillin	1.12	1.14	3.00	717	730	120
Difference	.49	.43	1.25	313	274	50

As seen from the table, the yield of the vanillin plot is far below the check plots. The average production of the two untreated plots was 1,030 pounds per acre of vines and 170 pecks of peas per acre,

while the vanillin plot produced 717 pounds per acre of vines and 120 pecks of peas. This is a reduction of 30 per cent in vines and 20 per cent in marketable peas, due to the presence of vanillin.

Plate IV, figure 2, shows the harvested crop grown in the untreated plot and in the vanillin plot.

EFFECT OF VANILLIN ON STRING BEANS.

String beans were also affected by vanillin. The beans were sown May 21, 1913; they germinated well and came up uniformly. The plants in the untreated plot grew better and were more thrifty than those in the vanillin plot. Plate III, figure 3, shows the comparative growth in the early stage, and from this it is seen that the untreated plants are much larger. The crop was harvested July 22. The beans were picked from the vines and measured. The results are given in Table IV.

TABLE IV.— *Yield of string beans as affected by vanillin in the field.*

Treatment.	Yield per plot.			Yield per acre.		
	Vines.	Beans.		Vines.	Beans.	
	Pounds.	*Pounds.*	*Pints.*	*Pounds.*	*Pounds.*	*Pecks.*
Check *a*	3.55	1.90	4.75	2,272	1,236	190
Check *b*	2.94	1.66	4.15	1,882	1,062	166
Average check	3.24	1.78	4.45	2,070	1,149	178
Vanillin	2.71	.55	1.50	1,734	352	56
Difference	.53	1.23	2.95	336	797	122

The average yield for the check plots was 2,070 pounds of vines per acre and 178 pecks of beans per acre. The yield of the vanillin plot was 1,734 pounds of vines per acre and 56 pecks of beans per acre. This is a decrease of 336 pounds of vines per acre and 122 pecks of beans per acre. The harvested crop from the untreated plot and the vanillin plot is shown in Plate IV, figure 3.

PRESENCE OF VANILLIN AND ITS EFFECT IN THE SOIL SIX MONTHS AFTER APPLICATION.

The question of the length of time the vanillin would persist in the Arlington soil and have an influence on its crop-producing power has also been investigated by a chemical study in the laboratory and by pot tests. Samples of soil for these purposes were obtained from the plots the last of November, six months after the substance was applied, and after a crop had been matured. The soils were examined for vanillin by the method already described by Shorey.[1] The method, in brief, consists of making an alkaline extract of the soil. The extract is acidified and filtered and then shaken out with ether.

[1] J. Agr. Research, **1**, 357 (1914).

The ether extract is shaken with a strong solution of sodium bisulphite, which treatment removes from the ether compounds of an aldehyde nature. After separating the bisulphite from the ether it is acidified with enough sulphuric acid completely to decompose it, is freed from sulphur dioxide by blowing air through it, and is again shaken with ether. The ether extract obtained by this process on evaporation gave an oily residue. The residues secured from the soils taken from the vanillin-treated plots which had grown cowpeas, garden peas, and string beans had the odor of vanillin. The residues were purified according to the method given in the paper cited. An aqueous solution of the purified residue from the three soils smelled strongly of vanillin. The aqueous solutions gave the color reactions characteristic of vanillin. Ferric chloride added to a portion of the solution gave a blue-violet color. When boiled with resorcinol and hydrochloric acid a red color resulted. The solution gave a violet color with a mixture of sulphuric and hydrochloric acid and acetone water. Bromine water and ferrous sulphate gave a green color. Or the addition of the reagent of Folin and Denis, the solution having been made alkaline with sodium carbonate, a clear blue color developed.

As is shown from the above examination that this vanillin-treated field soil still contained the substance, it was tested in pots as to its qualities for growing plants.

In this experiment wheat was grown in the greenhouse in paraffined wire pots, using the respective soils from the vanillin-treated plot and the check plots which in the field had grown cowpeas, garden peas, and string beans. The plants grew from December 11, 1913, to January 6, 1914. Two pots with 6 plants each were used for each soil. The results of the experiment are given in Table V.

TABLE V.—*Growth of wheat in pots of soil taken from the field plots six months after treatment with vanillin.*

Plot.	Green weight of wheat plants on—		
	Soil from plots untreated.	Soil from vanillin plots.	Relative growth, check = 100.
	Grams.	*Grams.*	
Cowpea plot	1.48	1.09	74
Garden pea plot	1.47	1.10	75
String bean plot	1.54	1.10	71

The table shows that the soils from the vanillin-treated plots were harmful to wheat in soil collected six months after the vanillin had been applied.

A similar experiment was made with these soils, except that the crops grown in the pots were identical with those which had grown in

the field the preceding season; that is, cowpeas on the cowpea soil from the check plot and from the vanillin plot, string beans on the string bean soil from both check and treated plots, garden peas on the garden pea soil from both check and treated plots. Two pots were used in each case and two plants in each pot. The plants grew from December 11 to January 6. The vegetative growth made in this experiment is given in Table VI.

TABLE VI.—*Growth of cowpeas in pots of soil from cowpea field plot; garden peas in soil from garden pea field plot; string beans in soil from string bean field plot; collected six months after treatment with vanillin.*

Plot.	Green weight of plants upon—		Relative growth, check=100.
	Soil from plots untreated.	Soil from vanillin plots.	
	Grams.	*Grams.*	
Cowpeas plot	4.30	3.05	71
Garden peas plot	5.60	4.00	71
String beans plot	7.80	7.35	94

The figures in the table show that vanillin was still harmful to the respective crops six months after the application of vanillin, and after it had produced the same crop in the field. These experiments show that vanillin persists in this heavy silty clay loam soil and affects its fertility for a considerable length of time.

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